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Tenth day of March 2003

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THE WALTER AND ELIZA HALL
INSTITUTE OF MEDICAL
RESEARCH

**AUSTRALIA**
**Patents Act 1990**

# PROVISIONAL SPECIFICATION
for the invention entitled:

"A NOVEL HAEMPOIETIN RECEPTOR AND GENETIC SEQUENCES ENCODING SAME - II"

The invention is described in the following statement:

# A NOVEL HAEMOPOIETIN RECEPTOR AND GENETIC SEQUENCES ENCODING SAME - II

The present invention relates generally to a novel haemopoietin receptor or components or parts thereof and to genetic sequences encoding same. The receptor molecules and their components and/or parts and the genetic sequences encoding same of the present invention are useful in the development of a wide range of agonists, antagonists, therapeutics and diagnostic reagents based on ligand interaction with its receptor.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The rapidly increasing sophistication of recombinant DNA techniques is greatly facilitating research into the medical and allied health fields. Cytokine research is of particular importance, especially as these molecules regulate the proliferation, differentiation and function of a wide variety of cells. Administration of recombinant cytokines or regulating cytokine function and/or synthesis is becoming increasingly the focus of medical research into the treatment of a range of disease conditions.

Despite the discovery of a range of cytokines and other secreted regulators of cell function, comparatively few cytokines are directly used or targeted in therapeutic regimums. One reason for this is the pleiotropic nature of many cytokines. For example, interleukin (IL)-11 is a functionally pleiotropic molecule (1,2), initially

characterized by its ability to stimulate proliferation of the IL-6-dependent plasmacytoma cell line, T11 65 (3). Other biological actions of IL-11 include induction of multipotential haemopoietin progenitor cell proliferation (4,5,6), enhancement of megakaryocyte and platelet formation (7,8,9,10), stimulation of acute phase protein synthesis (11) and inhibition of adipocyte lipoprotein lipase activity (12, 13).

Interleukin-13 (IL-13) is another important cytokine which shares a number of structural characteristics with interleukin-4 (IL-4) [reviewed in 14 and 15]. The genes for IL-4 and IL-13 have a related intron/exon structure and are located close together on chromosome 5 in the human and the syntonic region of chromosome 11 in the mouse (14, 15). At the protein level, IL-4 and IL-13 share approximately 30% amino acid identity, including four cysteine residues. Biologically, IL-13 and IL-4 are also similar, being produced by activated T-cells and acting upon macrophages to induce differentiation and suppress the production of inflammatory cytokines. Additionally, human IL-13 may act as a co-stimulatory signal for B-cell proliferation and affect immunoglobulin isotype switching (14, 15). The diverse and pleiotropic function of IL-13 and other haemopoietic cytokine makes this molecule an important group to study, especially at the level of interaction of the cytokine with its receptors. Manipulation and control of cytokine receptors and of cytokine-receptor interaction is potentially very important in many therapeutic situations, especially where the target cytokine is functionally pleiotropic and it is desired to block certain functions of a target cytokine but not all functions.

Research into IL-13 and its receptor has been hampered due to the inability to clone genetic sequences encoding all or part of the IL-13 receptor. In accordance with the present invention, genetic sequences have now been cloned encoding the IL-13 receptor α-chain. The availability of these genetic sequences permits the development of a range of therapeutic and diagnostic agents capable of modulating IL-13 activity as well as the activity of cytokines related at the level of IL-13 receptor structure.

Accordingly, one aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an haemopoietin receptor α-chain from an animal or a component, fragment, part, derivative, homologue or analogue thereof.

More particularly, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the IL-13 receptor α-chain from an animal or a component, fragment, part, derivative, homologue or analogue thereof.

In a related embodiment, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the IL-4 receptor α-chain from an animal or a component, fragment, part, derivative, homologue or analogue thereof.

Preferably, the animal is a mammal or a species of bird. Particularly, preferred mammals include humans, laboratory test animals (e.g. mice, rabbits, guinea pigs), livestock animals (e.g. sheep, horse, pigs, cows), companion animals (e.g. dogs, cats) or captive wild animals (e.g. kangaroos). Although the present invention is exemplified with respect to mice and humans, the scope of the subject invention extends to all animals and birds.

The present invention is predicated in part on an ability to identify members of the haemopoietin receptor family on the basis of sequence similarity. Based on this approach, a genetic sequence was identified in accordance with the present invention which encodes the IL-13 α-chain. The expressed genetic sequence is referred to herein as "NR4". NR4 has an apparent molecular weight when synthesised by transfected COS cells of from about 50,000 to about 70,000 daltons, and more preferably from about 55,000 to about 65,000 daltons. NR4 binds to IL-13 with low affinity and is considered, therefore, to be IL-13 receptor α-chain. Accordingly, the terms "NR4" and IL-13" receptor α-chain" (or "IL-13 Rα") are used interchangeably throughout the subject

specification. Furthermore, in accordance with the present invention, IL-13 binding to its receptor has been found to be competitively inhibited by IL-4 or a component thereof which may provide a method for controlling IL-13-receptor interaction and which may also provide a basis for the preparation and construction of mimetics.

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding IL-13 receptor α-chain having an amino acid sequence as set forth in SEQ ID NO:2 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

A further embodiment of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding the IL-13 receptor α-chain and having a nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

Still another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding IL-13 receptor α-chain having an amino acid sequence as set forth in SEQ ID NO:4 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

Yet still a further embodiment of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding the IL-13 receptor α-chain and having a nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

Accordingly, the present invention extends to the sequence of nucleotides set forth in SEQ ID NO:1 or 3 or the sequence of amino acids set forth in SEQ ID NO:2 or 4 or single or multiple nucleotide or amino acid substitutions, deletions and/or additions thereto.

The present invention further extends to nucleic acid molecules capable of hybridising under low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:1 or 3 or a complementary form thereof.

For the purposes of defining the level of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which are incorporated herein by reference where the washing step at paragraph 11 is considered herein to be high stringency. A low stringency wash is defined herein to be 0.1-0.2xSSC, 0.1% w/v SDS at 55-65°C for 20 minutes and a medium level of stringency is considered herein to be 2xSSC, 0.1% w/v SSC at ≥ 45°C for 20 minutes. The alternative conditions are applicable depending on concentration, purity and source of nucleic acid molecules.

Yet another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes an IL-13 receptor α-chain, said nucleic acid molecule having a nucleotide sequence substantially as set forth in SEQ ID NO:1 or 3 or a nucleic acid molecule which encodes a structurally similar IL-13 receptor α-chain or a derivative thereof and which is capable of hybridising to the nucleotide sequence substantially as set forth in SEQ ID NO:1 or 3 or a complementary form thereof under low stringency conditions.

Still yet another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes the IL-13 receptor α-chain having an amino acid sequence substantially as set forth in SEQ ID NO:2 or 4 or comprises a nucleotide sequence coding for an amino acid sequence having at least about 50% similarity to the sequence set forth in SEQ ID NO:2 or 4 and is capable of hybridising to the sequence set forth in SEQ ID NO:1 or 3 under low stringency conditions.

The nucleic acid molecules contemplated by the present invention are generally in isolated form and are preferably cDNA or genomic DNA molecules. In a particularly preferred embodiment, the nucleic acid molecules are in vectors and most preferably expression vectors to enable expression in a suitable host cell. Particularly useful host cells include prokaryotic cells, mammalian cells, yeast cells and insect cells. The cells may also be in the form of a cell line.

According to this aspect of the present invention there is provided an expression vector comprising a nucleic acid molecule encoding the IL-13 receptor α-chain as hereinbefore described, said expression vector capable of expression in a particularly host cell.

Another aspect of the present invention contemplates a recombinant polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:2 or 4 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

The recombinant polypeptide contemplated by the present invention includes, therefore, components, parts, fragments, derivatives, homologues or analogues of the IL-13 receptor α-chain and is preferably encoded by a nucleotide sequence substantially set forth in SEQ ID NO:1 or 3 or a molecule having at least about 50% similarity to all or part thereof or a molecule capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:1 or 3 or a complementary form thereof. The recombinant molecule may be glycosylated or non-glycosylated. When in glycosylated form, the glycosylation may be substantially the same as naturally occurring IL-13 receptor α-chain or may be a modified form of glycosylation. Altered or differential glycosylation states may or may not affect binding activity of the IL-13 receptor α-chain.

The recombinant IL-13 receptor α-chain may be in soluble form or may be expressed on a cell surface or conjugated or fused to a solid support or another molecule.

The present invention extends to chemical analogues of the recombinant IL-13 receptor α-chain.

Chemical analogues of the recombinant IL-13 receptor α-chain contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of $C_\alpha$ and $N_\alpha$-methylamino acids, introduction of double bonds between $C_\alpha$ and $C_\beta$ atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

Chemical modification of the recombinant IL-13 receptor $\alpha$-chain may be important, for example, to increase serum half-life, to protect the molecule from enzymatic degradation and/or for diagnostic purposes.

The recombinant IL-13 receptor $\alpha$-chain contemplated by the present invention is useful in the development of a range of agonists and antagonists of IL-13-receptor interaction. The recombinant molecule may also be used in the development of diagnostic agents.

Particularly useful agents encompassed by this aspect of the present invention are antibodies to the recombinant IL-13 receptor $\alpha$-chain. The antibodies may be monoclonal or polyclonal and are particularly useful as antagonists of IL-13-receptor binding or as diagnostic agents to qualitate or quantitate the presence of the IL-13 receptor $\alpha$-chain. These antibodies may also be useful in the screening of similar components in other receptors such as IL-4 receptors.

Other agonists and antagonists include chemical molecules which, for example, structurally, functionally or electrochemically mimic or have similarities to IL-13 receptor $\alpha$-chain or which comprise a solubilised form of the IL-13 receptor $\alpha$-chain.

Such agents are useful in modulating IL-13-receptor interaction and these are useful in enhancing or diminishing IL-13 related activities. This may be particularly important for cancers or tumours involving or resulting from excess IL-13 or from aberrant IL-13 molecules or to promote IL-13 function in the treatment of a range of conditions such as, but not limited to, immune deficiency.

The present invention further contemplates ribozyme and antisense molecules useful in reducing IL-13 receptor $\alpha$-chain expression.

The present invention encompasses, therefore, pharmaceutical and diagnostic compositions comprising recombinant IL-13 receptor $\alpha$-chain or parts thereof, antibodies thereto, agonists or antagonists thereof or genetic molecules such as ribozymes, antisense molecules and constructs useful in co-suppression.

The present invention is further described by the following non-limiting Figures and Examples.

In the Figures:

**Figure 1** is a representation of the nucleotide [SEQ ID NO:1] and predicted amino acid [SEQ ID NO:2] sequence of murine NR4. The untranslated region is shown in lower case and the translated region in upper case. The conventional one-letter code for amino acids is employed, potential asparagine linked glycosylation sites are underlined and the conserved cysteine residues and WSXWS motif of haempoietin receptor family members are shown in bold. The predicted signal sequence is underlined in bold while the transmembrane domain is underlined with dashes. The sequence shown is a composite derived from the analysis of 8 cDNA clones derived from 3 libraries. The 5′-end of the sequence (nucleotides -60 to 351) is derived from a single cDNA clone but is also present in genomic DNA clones that have been isolated.

**Figure 2** is a photographic representation showing northern analysis of murine NR4 mRNA expression in selected tissues and organs.

**Figure 3** is a graphical representation depicting saturation isotherms of $^{125}$I-IL-13 and $^{125}$I-IL-4 binding; saturation isotherms depicted as Scatchard plots of IL-4 ( o ) and IL-13 (•) binding to (A) COS cells expressing the IL-13Rα (NR4), (B) CTLL cells and (C) CTLL cells expressing the IL-13Rα (NR4). Data have been normalised to $1x10^4$ COS cells and $1x10^6$ CTLL cells and binding was carried out on ice for 2 to 4 hours.

**Figure 4** is a graphical representation showing specificity of IL-4 and IL-13 binding; the ability of IL-4 ( o ) and IL-13 (•) to compete for $^{125}$I-$^{125}$I-IL-13 binding to (A) COS cells expressing the IL-13Rα (NR4) and (C) CTLL cells expressing the IL-13Rα (NR4) or to compete for IL-4 binding to (B) CTLL cells and (D). CTLL cells expressing the IL-13Rα (NR4) binding was carried out on ice for 2 to 4 hours and the data have been expressed as a percentage of the specific binding observed in the absence of a competitor

(■).

**Figure 5** is a graphical representation showing factor dependent proliferation of cells expressing NR4. Two hundred (A) CTLL cells or (B) CTLL cells expressing the IL-13Rα (NR4) were incubated in the absence of cytokine (■) or with various concentrations of IL-2 (□), IL-4 ( ○ ) or IL-13 (•). After 48 hours viable cells were counted and data was expressed as a percentage of the number of viable cells observed with a maximal concentration of IL-2.

**Figure 6** is a photographic representation showing cross-species conservation of NR4 (IL-13Rα) gene.

**Figure 7** is a representation of the nucleotide and corresponding amino acid sequence of murine and human NR4 (IL-13Rα) genes. The nucleotide and predicted amino acid sequence of human (H) and murine (M) IL-13Rα (NR4) were aligned by eye, with gaps (-) inserted to optimise the alignment. The numbering is for the murine clone, nucleotides that form part of the coding region are shown in upper case, whilst those of the untranslated regions are shown in lower case. Amino acids identical between the predicted murine and human proteins are indicated by (*). DNA encoding the murine signal sequence is underlined, with A26 or T27 being the predicted first amino acid of the mature protein.

**Figure 8** is a photographic representation showing NR4 expression in mouse tissues.

The following single and three letter abbreviations for amino acid residues are used in the specification:

| Amino Acid | Three-letter Abbreviation | One-letter Symbol |
|---|---|---|
| Alanine | Ala | A |
| Arginine | Arg | R |
| Asparagine | Asn | N |
| Aspartic acid | Asp | D |
| Cysteine | Cys | C |
| Glutamine | Gln | Q |
| Glutamic acid | Glu | E |
| Glycine | Gly | G |
| Histidine | His | H |
| Isoleucine | Ile | I |
| Leucine | Leu | L |
| Lysine | Lys | K |
| Methionine | Met | M |
| Phenylalanine | Phe | F |
| Proline | Pro | P |
| Serine | Ser | S |
| Threonine | Thr | T |
| Tryptophan | Trp | W |
| Tyrosine | Tyr | Y |
| Valine | Val | V |
| Any residue | Xaa | X |

## EXAMPLE 1

### Isolation of genomic and cDNAs encoding NR4

*Apo*I digested genomic DNA, extracted from an embryonal stem cell line, was cloned into the λZAPII bacteriophage (Stratagene, LaJolla, CA). Approximately $10^6$ plaques from this library were screened with a $^{32}$P-labelled oligonucleotide corresponding to the sequence Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] (16). Positively hybridising clones were sequenced using an automated DNA sequencer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). One clone appeared to encode for part of a new member of the haemopoietin receptor family. Oligonucleotides were designed on the basis of this genomic DNA sequence and were used in the conventional manner to isolate clones from mouse peritoneal macrophage (Clontech Laboratories, Palo Alto, CA), mouse skin, mouse lung, mouse kidney, and WEHI-3B (Stratagene, LaJolla, CA) λ-bacteriophage cDNA libraries.

## EXAMPLE 2

### Construction of expression vectors and transfection of cells

Using PCR, a derivative of the NR4 cDNA was generated which encoded for the IL-3 signal sequence and an N-terminal FLAG epitope-tag preceding the mature coding region of NR4 (Thr27 to Pro424; Figure 1). The PCR product was cloned into the mammalian expression vector pEF-BOS (17). Constructs were sequenced in their entirety prior to use. Cells were transfected and selected as previously described (16, 18).

## EXAMPLE 3

### Northern blots

Northern blots were performed as previously described (16). The source of hybridisation probes was as follows: NR4 - a PCR product from nucleotide 32 to 984 (Figure 1) and GAPDH - a cDNA fragment spanning nucleotides (19) [REF REQUIRED].

## EXAMPLE 4

**Cytokines and binding experiments using radioiodinated cytokines**

IL-2, IL-4, IL-7, IL-9, IL-13 and IL-15 were obtained commercially (R & D Systems, Minneapolis MN). For radioiodination, cytokines were dissolved at a concentration of 100 μg/ml in 10 mM sodium phosphate, 150 mM NaCl (PBS), 0.02% v/v Tween 20 and 0.02% w/v sodium azide at pH 7.4. An amount of 2μg of IL-13 was radioiodinated using the iodine monochloride method (20, 21), while 2μg of IL-4 was radiolabelled using dioiodo-Bolton-Hunter reagent (16). Binding studies and determination of the specific radioactivity and bindability of labelled cytokines were performed as previously described (2).

## EXAMPLE 5

**Proliferation Assays**

The proliferation of Ba/F3 and CTLL cells in response to cytokines was measured in Lux 60 microwell HL-A plates (Nunc Inc. IL, USA). Cells were washed three times in DME containing 20% v/v new born calf serum and resuspended at a concentration of $2 \times 10^4$ cells per ml in the same medium. Aliquots of 10μl of the cell suspension were placed in the culture wells with 5μl of various concentrations of purified recombinant cytokines. After 2 days of incubation at 37°C in a fully humidified incubator containing 10% v/v $CO_2$ in air, viable cells were counted using an inverted microscope.

## EXAMPLE 6

**Cloning and Characterisation of Murine NR4**

A library was constructed in λZAPII using *Apo*I digested genomic DNA from embryonal stem cells and screened with a pool of $^{32}$P-labelled oligonucleotides encoding the amino acid sequence Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] found in many members of the haemopoietin receptor family. One hybridising bacteriophage was found to contain a genomic clone that appeared to encode part of a novel member of the haemopoietin receptor family. This receptor was given the operational name NR4. The sequence of the genomic clone was used to isolate cDNAs encoding NR4 from WEHI-3B cell, peritoneal macrophage, bone marrow, skin and kidney libraries. A composite of the

nucleotide sequence [SEQ ID NO:1] and predicted amino acid sequence [SEQ ID NO:2] of these cDNAs is shown in Figure 1. The NR4 cDNA is predicted to encode for a protein of 424 amino acid residues, containing a putative signal sequence and transmembrane domain. The extracellular region of the protein containing a putative signal sequence and transmembrane domain. The extracellular region of the protein contained an immunoglobulin-like domain (amino acids 27-117), in addition to a typical haempoietin receptor domain (amino acids 118-340) which includes four conserved cysteine residues and the characteristic Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] motif (Figure 1). The cytoplasmic tail of the new receptor was 60 amino acids in length.

## EXAMPLE 7

### Expression pattern of NR4 cDNA

The pattern of NR4 mRNA expression was examined by Northern analyses. Two hybridising species of 5.2 and 2.2 kb in length were detected in mRNA from most tissues (Figure 2). NR4 mRNA was not detectable in skeletal muscle (Figure 2). Figure 8 shows expression of NR4 in mouse tissues.

## EXAMPLE 8

### NR4 encodes the IL-13 receptor α-chain (IL-13Rα) - a specific binding subunit of the IL-13 receptor

The apparent molecular weight is from about 50,000 to about 70,000 daltons and more particularly about 55,000 to about 65,000 daltons for NR4 expressed in COS cells estimated from Western blots using an anti-FLAG antibody, suggested that NR4 might encode the binding subunit of the IL-13 receptor. In order to test this possibility NR4 was expressed in COS cells. Untransfected COS cells expressed relatively low levels of IL-4 and IL-13 receptors. Upon transfection with a plasmid containing the NR4 cDNA, the number of IL-13 receptors but not IL-4 receptors expressed by COS cells was dramatically increased (Figure 3A; 100,000 to 500,000 receptors per cell). The affinity of IL-13 for NR4 expressed by COS cells was low ($K_D$~2-10 nM) and binding was specific since it was in competition with unlabelled IL-13 but not other cytokines including IL-2, IL-4, IL-7, IL-9 or IL-15 (Figure 4A). These results suggest that NR4 is the IL-13 receptor α-chain (IL-13Rα).

## EXAMPLE 9

### The IL-13Rα (NR4) and the IL-4Rα are shared components of the IL-4 and IL-3 receptors

In order to investigate the relationship between IL-4 and IL-13 receptors, the IL-4 responsive cell line CTLL was examined. Parental CTLL cells expressed a single class of IL-4 receptor ($K_D$~660 pM; ~3600 receptors per cell) but no detectable IL-13 receptors (Figure 3B). The IL-4 receptors expressed by CTLL cells appeared to be specific since binding of $^{125}$I-IL-4 was in competition with unlabelled IL-4 but not IL-13 (Figure 4B). Upon expression of the IL-13Rα (NR4) in CTLL cells no change was observed in the number or affinity of IL-4 receptors, while a single class of high affinity IL-13 receptors was detected (Figure 3C; $K_D$~75 pM; 1350 receptors per cell). The affinity of IL-13 for the IL-13Rα (NR4) expressed in CTLL cells was higher than in COS cells, suggesting that the former expressed a protein capable of interacting with the IL-13Rα (NR4) to increase the affinity for IL-13. A likely candidate based on previous studies is the IL-4Rα. In order to explore this possibility the ability of IL-4 to compete with $^{125}$I-IL-13 for binding to CTLL cells expressing the IL-13Rα (NR4) was assessed. Figure 4B shows that IL-4 and IL-13 were equally effective in competing for $^{125}$I-IL-13 binding ($IC_{50}$ ~ 300pM; Figure 4C) and, in addition, were able to compete with $^{125}$I-IL-4 for binding ($IC_{50}$ ~ 300 pm; Figure 4D).

## EXAMPLE 10

### Expression of the IL-13Rα (NR4) is necessary for transduction of a proliferative signal by IL-13

CTLL cells require the addition of exogenous cytokines for survival and proliferation. IL-2 was found to be a potent proliferative stimulus for CTLL cells ($EC_{50}$ ~ 100-200 pM), while IL-4 was relatively weak ($EC_{50}$ 2-7 nM) and IL-13 was inactive (Figure 5A). Expression of the IL-13Ra (NR4) in CTLL cells resulted in the ability to survive and proliferate weakly in response to IL-13 ($EC_{50}$ ~ 700 pM) and to proliferate somewhat more strongly than parental cells in response to IL-4 ($EC_{50}$ ~ 700 pM; Figure 5B).

## EXAMPLE 11

### Cloning of Human IL-13Rα (NR4)

In order to determine whether genes homologous to murine IL-13Rα (NR4) exist in other vertebrate species, a probe encompassing nucleotides 840 to 1270 of murine IL-13Rα (NR4) was hybridised to *Eco*RI digested genomic DNA from various species. Hybridisation was carried out in 500 mM $Na_2HPO_4$ (~5xSSC) at 50°C overnight. The filter was washed in 40 mM $Na_2HPO_4$ (~0.2xSSC) at 50°C for 2 hours and exposed to autoradiographic film for 48 hours. Figure 6 illustrates that relatively few (1 to 5) hybridising bands are observed in genomic DNA from various species, including human. This suggests that it is feasible to clone human IL-13Rα (NR4) using a murine cDNA probe. A human bone marrow cDNA library clones in the λZAPII bacteriophage was therefore screened with two probes (nucleotides 82-840 and 840 to 1270) from the murine IL-13Rα (NR4) cDNA. Hybridisation was carried out overnight in 6xSSC, 0.1% w/v SDS at 42°C. Filters were washed at 2xSSC, 0.1% w/v SDS at 50°C for 2 hours and exposed for 48 hours to autoradiographic film. Plaques that hybridised to both murine IL-13Rα (NR4) probes were picked and purified in the conventional manner. The cDNA inserts form the hybridising bacteriophage were excised into the pBluescript plasmid and sequenced in their entirety using an ABI automated sequencer. Figure 7 shows a composite of the sequence of the clones isolated and reveals that the clones encode a protein that shares a high degree of sequence similarity with murine IL-13Rα (NR4). The clones encode for the entire mature coding region of the protein, but lack the initiation methionine and the signal sequence; the high degree of sequence similarity (311/401 amino acids ~ 78%) predicates that this cDNA is the human homologue of the murine IL-13Rα (NR4).

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH

(ii) TITLE OF INVENTION: A NOVEL HAEMOPOIETIN RECEPTOR AND GENETIC SEQUENCES ENCODING SAME - II

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: AU PROVISIONAL
(B) FILING DATE: 23-OCT-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HUGHES DR, E JOHN L
(C) REFERENCE/DOCKET NUMBER: EJH/EK

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +61 3 9254 2777
(B) TELEFAX: +61 3 9254 2770

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1680 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1272

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TGAAAAGATA GAATAAATGG CCTCGTGCCG AATTCGGCAC GAGCCGAGGC GAGGGCCTGC        -1

ATG GCG CGG CCA GCG CTG CTG GGC GAG CTG TTG GTG CTG CTA CTG TGG          48
Met Ala Arg Pro Ala Leu Leu Gly Glu Leu Leu Val Leu Leu Leu Trp
 1               5                  10                  15

ACC GCC ACC GTG GGC CAA GTT GCC GCG GCC ACA GAA GTT CAG CCA CCT          96
Thr Ala Thr Val Gly Gln Val Ala Ala Ala Thr Glu Val Gln Pro Pro
            20                  25                  30

GTG ACG AAT TTG AGC GTC TCT GTC GAA AAT CTC TGC ACG ATA ATA TGG         144
Val Thr Asn Leu Ser Val Ser Val Glu Asn Leu Cys Thr Ile Ile Trp
        35                  40                  45

ACG TGG AGT CCT CCT GAA GGA GCC AGT CCA AAT TGC ACT CTC AGA TAT         192
Thr Trp Ser Pro Pro Glu Gly Ala Ser Pro Asn Cys Thr Leu Arg Tyr
    50                  55                  60

TTT AGT CAC TTT GAT GAC CAA CAG GAT AAG AAA ATT GCT CCA GAA ACT         240
Phe Ser His Phe Asp Asp Gln Gln Asp Lys Lys Ile Ala Pro Glu Thr
65                  70                  75                  80

CAT CGT AAA GAG GAA TTA CCC CTG GAT GAG AAA ATC TGT CTG CAG GTG         288
His Arg Lys Glu Glu Leu Pro Leu Asp Glu Lys Ile Cys Leu Gln Val
                85                  90                  95

GGC TCT CAG TGT AGT GCC AAT GAA AGT GAG AAG CCT AGC CCT TTG GTG         336
Gly Ser Gln Cys Ser Ala Asn Glu Ser Glu Lys Pro Ser Pro Leu Val
            100                 105                 110

AAA AAG TGC ATC TCA CCC CCT GAA GGT GAT CCT GAG TCC GCT GTG ACT         384
Lys Lys Cys Ile Ser Pro Pro Glu Gly Asp Pro Glu Ser Ala Val Thr
        115                 120                 125

GAG CTC AAG TGC ATT TGG CAT AAC CTG AGC TAT ATG AAG TGT TCC TGG         432
Glu Leu Lys Cys Ile Trp His Asn Leu Ser Tyr Met Lys Cys Ser Trp
    130                 135                 140

CTC CCT GGA AGG AAT ACA AGC CCT GAC ACA CAC TAT ACT CTG TAC TAT         480
Leu Pro Gly Arg Asn Thr Ser Pro Asp Thr His Tyr Thr Leu Tyr Tyr
145                 150                 155                 160

TGG TAC AGC AGC CTG GAG AAA AGT CGT CAA TGT GAA AAC ATC TAT AGA         528
Trp Tyr Ser Ser Leu Glu Lys Ser Arg Gln Cys Glu Asn Ile Tyr Arg
                165                 170                 175

GAA GGT CAA CAC ATT GCT TGT TCC TTT AAA TTG ACT AAA GTG GAA CCT         576
Glu Gly Gln His Ile Ala Cys Ser Phe Lys Leu Thr Lys Val Glu Pro
            180                 185                 190

AGT TTT GAA CAT CAG AAC GTT CAA ATA ATG GTC AAG GAT AAT GCT GGG         624
Ser Phe Glu His Gln Asn Val Gln Ile Met Val Lys Asp Asn Ala Gly
        195                 200                 205
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AAA ATT AGG CCA TCC TGC AAA ATA GTG TCT TTA ACT TCC TAT GTG AAA       672
Lys Ile Arg Pro Ser Cys Lys Ile Val Ser Leu Thr Ser Tyr Val Lys
    210                 215                 220

CCT GAT CCT CCA CAT ATT AAA CAT CTT CTC CTC AAA AAT GGT GCC TTA       720
Pro Asp Pro Pro His Ile Lys His Leu Leu Leu Lys Asn Gly Ala Leu
225                 230                 235                 240

TTA GTG CAG TGG AAG AAT CCA CAA AAT TTT AGA AGC AGA TGC TTA ACT       768
Leu Val Gln Trp Lys Asn Pro Gln Asn Phe Arg Ser Arg Cys Leu Thr
                245                 250                 255

TAT GAA GTG GAG GTC AAT AAT ACT CAA ACC GAC CGA CAT AAT ATT TTA       816
Tyr Glu Val Glu Val Asn Asn Thr Gln Thr Asp Arg His Asn Ile Leu
            260                 265                 270

GAG GTT GAA GAG GAC AAA TGC CAG AAT TCC GAA TCT GAT AGA AAC ATG       864
Glu Val Glu Glu Asp Lys Cys Gln Asn Ser Glu Ser Asp Arg Asn Met
        275                 280                 285

GAG GGT ACA AGT TGT TTC CAA CTC CCT GGT GTT CTT GCC GAC GCT GTC       912
Glu Gly Thr Ser Cys Phe Gln Leu Pro Gly Val Leu Ala Asp Ala Val
    290                 295                 300

TAC ACA GTC AGA GTA AGA GTC AAA ACA AAC AAG TTA TGC TTT GAT GAC       960
Tyr Thr Val Arg Val Arg Val Lys Thr Asn Lys Leu Cys Phe Asp Asp
305                 310                 315                 320

AAC AAA CTG TGG AGT GAT TGG AGT GAA GCA CAG AGT ATA GGT AAG GAG      1008
Asn Lys Leu Trp Ser Asp Trp Ser Glu Ala Gln Ser Ile Gly Lys Glu
                325                 330                 335

CAA AAC TCC ACC TTC TAC ACC ACC ATG TTA CTC ACC ATT CCA GTC TTT      1056
Gln Asn Ser Thr Phe Tyr Thr Thr Met Leu Leu Thr Ile Pro Val Phe
            340                 345                 350

GTC GCA GTG GCA GTC ATA ATC CTC CTT TTT TAC CTG AAA AGG CTT AAG      1104
Val Ala Val Ala Val Ile Ile Leu Leu Phe Tyr Leu Lys Arg Leu Lys
        355                 360                 365

ATC ATT ATA TTT CCT CCA ATT CCT GAT CCT GGC AAG ATT TTT AAA GAA      1152
Ile Ile Ile Phe Pro Pro Ile Pro Asp Pro Gly Lys Ile Phe Lys Glu
    370                 375                 380

ATG TTT GGA GAC CAG AAT GAT GAT ACC CTG CAC TGG AAG AAG TAT GAC      1200
Met Phe Gly Asp Gln Asn Asp Asp Thr Leu His Trp Lys Lys Tyr Asp
385                 390                 395                 400

ATC TAT GAG AAA CAA TCC AAA GAA GAA ACG GAT TCT GTA GTG CTG ATA      1248
Ile Tyr Glu Lys Gln Ser Lys Glu Glu Thr Asp Ser Val Val Leu Ile
                405                 410                 415

GAA AAC CTG AAG AAA GCA GCT CCT TGATGGGGAG AAGTGATTTC TTTCTTGCCT     1302
Glu Asn Leu Lys Lys Ala Ala Pro
            420

TCAATGTGAC CCTGTGAAGA TTTATTGCAT TCTCCATTTG TTATCTGGGG GACTTGTTAA    1362

ATAGAAACTG AAACTACTCT TGAAAAACAG GCAGCTCCTA AGAGCCACAG GTCTTGATGT    1422

GACTTTTGCA TTGAAAACCC AAACCCAAAG GAGCTCCTTC CAAGAAAAGC AAGAGTTCTT    1482

CTCGTTCCTT GTTCCAATCC CTAAAAGCAG ATGTTTTGCC AAATCCCCAA ACTAGAGGAC    1542

AAAGACAAGG GGACAATGAC CATCAATTCA TCTAATCAGG AATTGTGATG GCTTCCTAAG    1602

GAATCTCTGC TTGCTCTG                                                  1620
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 424 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Arg Pro Ala Leu Leu Gly Glu Leu Leu Val Leu Leu Leu Trp
  1               5                  10                  15

Thr Ala Thr Val Gly Gln Val Ala Ala Ala Thr Glu Val Gln Pro Pro
             20                  25                  30

Val Thr Asn Leu Ser Val Ser Val Glu Asn Leu Cys Thr Ile Ile Trp
         35                  40                  45

Thr Trp Ser Pro Pro Glu Gly Ala Ser Pro Asn Cys Thr Leu Arg Tyr
     50                  55                  60

Phe Ser His Phe Asp Asp Gln Gln Asp Lys Lys Ile Ala Pro Glu Thr
 65                  70                  75                  80

His Arg Lys Glu Glu Leu Pro Leu Asp Glu Lys Ile Cys Leu Gln Val
                 85                  90                  95

Gly Ser Gln Cys Ser Ala Asn Glu Ser Glu Lys Pro Ser Pro Leu Val
            100                 105                 110

Lys Lys Cys Ile Ser Pro Pro Glu Gly Asp Pro Glu Ser Ala Val Thr
        115                 120                 125

Glu Leu Lys Cys Ile Trp His Asn Leu Ser Tyr Met Lys Cys Ser Trp
    130                 135                 140

Leu Pro Gly Arg Asn Thr Ser Pro Asp Thr His Tyr Thr Leu Tyr Tyr
145                 150                 155                 160

Trp Tyr Ser Ser Leu Glu Lys Ser Arg Gln Cys Glu Asn Ile Tyr Arg
                165                 170                 175

Glu Gly Gln His Ile Ala Cys Ser Phe Lys Leu Thr Lys Val Glu Pro
            180                 185                 190

Ser Phe Glu His Gln Asn Val Gln Ile Met Val Lys Asp Asn Ala Gly
        195                 200                 205

Lys Ile Arg Pro Ser Cys Lys Ile Val Ser Leu Thr Ser Tyr Val Lys
    210                 215                 220

Pro Asp Pro Pro His Ile Lys His Leu Leu Leu Lys Asn Gly Ala Leu
225                 230                 235                 240

Leu Val Gln Trp Lys Asn Pro Gln Asn Phe Arg Ser Arg Cys Leu Thr
                245                 250                 255

Tyr Glu Val Glu Val Asn Asn Thr Gln Thr Asp Arg His Asn Ile Leu
            260                 265                 270

Glu Val Glu Glu Asp Lys Cys Gln Asn Ser Glu Ser Asp Arg Asn Met
        275                 280                 285

Glu Gly Thr Ser Cys Phe Gln Leu Pro Gly Val Leu Ala Asp Ala Val
    290                 295                 300

Tyr Thr Val Arg Val Arg Val Lys Thr Asn Lys Leu Cys Phe Asp Asp
305                 310                 315                 320
```

```
Asn Lys Leu Trp Ser Asp Trp Ser Glu Ala Gln Ser Ile Gly Lys Glu
                325                 330                 335

Gln Asn Ser Thr Phe Tyr Thr Thr Met Leu Leu Thr Ile Pro Val Phe
            340                 345                 350

Val Ala Val Ala Val Ile Ile Leu Leu Phe Tyr Leu Lys Arg Leu Lys
        355                 360                 365

Ile Ile Ile Phe Pro Pro Ile Pro Asp Pro Gly Lys Ile Phe Lys Glu
    370                 375                 380

Met Phe Gly Asp Gln Asn Asp Asp Thr Leu His Trp Lys Lys Tyr Asp
385                 390                 395                 400

Ile Tyr Glu Lys Gln Ser Lys Glu Glu Thr Asp Ser Val Val Leu Ile
                405                 410                 415

Glu Asn Leu Lys Lys Ala Ala Pro
            420
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1248 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1203

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCG CCT ACG GAA ACT CAG CCA CCT GTG ACA AAT TTG AGT GTC TCT GTT 48
Ala Pro Thr Glu Thr Gln Pro Pro Val Thr Asn Leu Ser Val Ser Val
1 5 10 15

GAA AAC CTC TGC ACA GTA ATA TGG ACA TGG AAT CCA CCC GAG GGA GCC 96
Glu Asn Leu Cys Thr Val Ile Trp Thr Trp Asn Pro Pro Glu Gly Ala
20 25 30

AGC TCA AAT TGT AGT CTA TGG TAT TTT AGT CAT TTT GGC GAC AAA CAA 144
Ser Ser Asn Cys Ser Leu Trp Tyr Phe Ser His Phe Gly Asp Lys Gln
35 40 45

GAT AAG AAA ATA GCT CCG GAA ACT CGT CGT TCA ATA GAA GTA CCC CTG 192
Asp Lys Lys Ile Ala Pro Glu Thr Arg Arg Ser Ile Glu Val Pro Leu
50 55 60

AAT GAG AGG ATT TGT CTG CAA GTG GGG TCC CAG TGT AGC ACC AAT GAG 240
Asn Glu Arg Ile Cys Leu Gln Val Gly Ser Gln Cys Ser Thr Asn Glu
65 70 75 80

AGT GAG AAG CCT AGC ATT TTG GTT GAA AAA TGC ATC TCA CCC CCA GAA 288
Ser Glu Lys Pro Ser Ile Leu Val Glu Lys Cys Ile Ser Pro Pro Glu
85 90 95

GGT GAT CCT GAG TCT GCT GTG ACT GAA CTT CAA TGC ATT TGG CAC AAC 336
Gly Asp Pro Glu Ser Ala Val Thr Glu Leu Gln Cys Ile Trp His Asn
100 105 110

CTG AGC TAC ATG AAG TGT TCT TGG CTC CCT GGA AGG AAT ACC AGT CCC 384
Leu Ser Tyr Met Lys Cys Ser Trp Leu Pro Gly Arg Asn Thr Ser Pro
115 120 125

GAC ACT AAC TAT ACT CTC TAC TAT TGG CAC AGA AGC CTG GAA AAA ATT 432
Asp Thr Asn Tyr Thr Leu Tyr Tyr Trp His Arg Ser Leu Glu Lys Ile
130 135 140

CAT CAA TGT GAA AAC ATC TTT AGA GAA GGC CAA TAC TTT GGT TGT TCC 480
His Gln Cys Glu Asn Ile Phe Arg Glu Gly Gln Tyr Phe Gly Cys Ser
145 150 155 160

TTT GAT CTG ACC AAA GTG AAG GAT TCC AGT TTT GAA CAA CAC AGT GTC 528
Phe Asp Leu Thr Lys Val Lys Asp Ser Ser Phe Glu Gln His Ser Val
165 170 175

CAA ATA ATG GTC AAG GAT AAT GCA GGA AAA ATT AAA CCA TCC TTC AAT 576
Gln Ile Met Val Lys Asp Asn Ala Gly Lys Ile Lys Pro Ser Phe Asn
180 185 190

ATA GTG CCT TTA ACT TCC CGT GTG AAA CCT GAT CCT CCA CAT ATT AAA 624
Ile Val Pro Leu Thr Ser Arg Val Lys Pro Asp Pro Pro His Ile Lys
195 200 205

AAC CTC TCC TTC CAC AAT GAT GAC CTA TAT GTG CAA TGG GAG AAT CCA 672
Asn Leu Ser Phe His Asn Asp Asp Leu Tyr Val Gln Trp Glu Asn Pro
210 215 220

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CAG AAT TTT ATT AGC AGA TGC CTA TTT TAT GAA GTA GAA GTC AAT AAC      720
Gln Asn Phe Ile Ser Arg Cys Leu Phe Tyr Glu Val Glu Val Asn Asn
225                 230                 235                 240

AGC CAA ACT GAG ACA CAT AAT GTT TTC TAC GTC CAA GAG GCT AAA TGT      768
Ser Gln Thr Glu Thr His Asn Val Phe Tyr Val Gln Glu Ala Lys Cys
                245                 250                 255

GAG AAT CCA GAA TTT GAG AGA AAT GTG GAG AAT ACA TCT TGT TTC ATG      816
Glu Asn Pro Glu Phe Glu Arg Asn Val Glu Asn Thr Ser Cys Phe Met
            260                 265                 270

GTC CCT GGT GTT CTT CCT GAT ACT TTG AAC ACA GTC AGA ATA AGA GTC      864
Val Pro Gly Val Leu Pro Asp Thr Leu Asn Thr Val Arg Ile Arg Val
        275                 280                 285

AAA ACA AAT AAG TTA TGC TAT GAG GAT GAC AAA CTC TGG AGT AAT TGG      912
Lys Thr Asn Lys Leu Cys Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp
    290                 295                 300

AGC CAA GAA ATG AGT ATA GGT AAG AAG CGC AAT TCC ACA CTC TAC ATA      960
Ser Gln Glu Met Ser Ile Gly Lys Lys Arg Asn Ser Thr Leu Tyr Ile
305                 310                 315                 320

ACC ATG TTA CTC ATT GTT CCA GTC ATC GTC GCA GGT GCA ATC ATA GTA     1008
Thr Met Leu Leu Ile Val Pro Val Ile Val Ala Gly Ala Ile Ile Val
                325                 330                 335

CTC CTG CTT TAC CTA AAA AGG CTC AAG ATT ATT ATA TTC CCT CCA ATT     1056
Leu Leu Leu Tyr Leu Lys Arg Leu Lys Ile Ile Ile Phe Pro Pro Ile
            340                 345                 350

CCT GAT CCT GGC AAG ATT TTT AAA GAA ATG TTT GGA GAC CAG AAT GAT     1104
Pro Asp Pro Gly Lys Ile Phe Lys Glu Met Phe Gly Asp Gln Asn Asp
        355                 360                 365

GAT ACT CTG CAC TGG AAG AAG TAC GAC ATC TAT GAG AAG CAA ACC AAG     1152
Asp Thr Leu His Trp Lys Lys Tyr Asp Ile Tyr Glu Lys Gln Thr Lys
    370                 375                 380

GAG GAA ACC GAC TCT GTA GTG CTG ATA GAA AAC CTG AAG AAA GCC TCT     1200
Glu Glu Thr Asp Ser Val Val Leu Ile Glu Asn Leu Lys Lys Ala Ser
385                 390                 395                 400

CAG TGA TGG AGA TAA TTT ATT TTT ACC TTC ACT GTG ACC TTG AGA AGA     1248
Gln
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 401 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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 1               5                  10                  15

Glu Asn Leu Cys Thr Val Ile Trp Thr Trp Asn Pro Pro Glu Gly Ala
            20                  25                  30

Ser Ser Asn Cys Ser Leu Trp Tyr Phe Ser His Phe Gly Asp Lys Gln
        35                  40                  45

Asp Lys Lys Ile Ala Pro Glu Thr Arg Arg Ser Ile Glu Val Pro Leu
    50                  55                  60

Asn Glu Arg Ile Cys Leu Gln Val Gly Ser Gln Cys Ser Thr Asn Glu
65                  70                  75                  80

Ser Glu Lys Pro Ser Ile Leu Val Glu Lys Cys Ile Ser Pro Pro Glu
                85                  90                  95

Gly Asp Pro Glu Ser Ala Val Thr Glu Leu Gln Cys Ile Trp His Asn
            100                 105                 110

Leu Ser Tyr Met Lys Cys Ser Trp Leu Pro Gly Arg Asn Thr Ser Pro
        115                 120                 125

Asp Thr Asn Tyr Thr Leu Tyr Tyr Trp His Arg Ser Leu Glu Lys Ile
    130                 135                 140

His Gln Cys Glu Asn Ile Phe Arg Glu Gly Gln Tyr Phe Gly Cys Ser
145                 150                 155                 160

Phe Asp Leu Thr Lys Val Lys Asp Ser Ser Phe Glu Gln His Ser Val
                165                 170                 175

Gln Ile Met Val Lys Asp Asn Ala Gly Lys Ile Lys Pro Ser Phe Asn
            180                 185                 190

Ile Val Pro Leu Thr Ser Arg Val Lys Pro Asp Pro Pro His Ile Lys
        195                 200                 205

Asn Leu Ser Phe His Asn Asp Asp Leu Tyr Val Gln Trp Glu Asn Pro
    210                 215                 220

Gln Asn Phe Ile Ser Arg Cys Leu Phe Tyr Glu Val Glu Val Asn Asn
225                 230                 235                 240

Ser Gln Thr Glu Thr His Asn Val Phe Tyr Val Gln Glu Ala Lys Cys
                245                 250                 255

Glu Asn Pro Glu Phe Glu Arg Asn Val Glu Asn Thr Ser Cys Phe Met
            260                 265                 270

Val Pro Gly Val Leu Pro Asp Thr Leu Asn Thr Val Arg Ile Arg Val
        275                 280                 285

Lys Thr Asn Lys Leu Cys Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp
    290                 295                 300

Ser Gln Glu Met Ser Ile Gly Lys Lys Arg Asn Ser Thr Leu Tyr Ile
305                 310                 315                 320
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Thr Met Leu Leu Ile Val Pro Val Ile Val Ala Gly Ala Ile Ile Val
                325                 330                 335

Leu Leu Leu Tyr Leu Lys Arg Leu Lys Ile Ile Ile Phe Pro Pro Ile
            340                 345                 350

Pro Asp Pro Gly Lys Ile Phe Lys Glu Met Phe Gly Asp Gln Asn Asp
        355                 360                 365

Asp Thr Leu His Trp Lys Lys Tyr Asp Ile Tyr Glu Lys Gln Thr Lys
    370                 375                 380

Glu Glu Thr Asp Ser Val Val Leu Ile Glu Asn Leu Lys Lys Ala Ser
385                 390                 395                     400

Gln
```

DATED this 22nd day of December, 1995

THE WALTER AND ELIZA HALL INSTITUTE
OF MEDICAL RESEARCH
By Its Patent Attorneys
DAVIES COLLISON CAVE

```
-60   [illegible]
      [illegible]
1     M  A  R  F  A  L  L  G  E  L  L  V  L  L  L  W  T  A  T  V

61    GGCCAAGTTGCCGCGGCCACAGAAGTTCAGCCACCTGTGACGAATTTGAGCCTCTCTGTC
21    G  Q  V  A  A  A  T  E  V  Q  P  P  V  T  N  L  S  V  S  V

121   GAAAATCTCTGCACGATAATATGGACGTGGAGTCCTCCTGAAGGAGCCAGTCCAAATTGC
41    E  N  L  C  T  I  I  W  T  W  S  P  P  E  G  A  S  P  N  C

181   ACTCTCAGATATTTTAGTCACTTTGATGACCAACAGGATAAGAAAATTGCTCCAGAAACT
61    T  L  R  Y  F  S  H  F  D  D  Q  Q  D  K  K  I  A  P  E  T

241   CATCGTAAACACGAATTACCCCTGGATGAGAAAATCTGTCTGCAGGTGGGCTCTCAGTGT
81    H  R  K  E  E  L  P  L  D  E  K  I  C  L  Q  V  G  S  Q  C

301   AGTGCCAATGAAAGTGAGAAGCCTAGCCCTTTGGTGAAAAAGTGCATCTCACCCCCTGAA
101   S  A  N  E  S  E  K  P  S  P  L  V  K  K  C  I  S  P  P  E

361   GGTGATCCTGAGTCCGCTGTGACTGAGCTCAAGTGCATTTGGCATAACCTGAGCTATATG
121   G  D  P  E  S  A  V  T  E  L  K  C  I  W  H  N  L  S  Y  M

421   AAGTGTTCCTGGCTCCCTGGAAGGAATACAAGCCCTGACACACACTATACTCTGTACTAT
141   K  C  S  W  L  P  G  R  N  T  S  P  D  T  H  Y  T  L  Y  Y

481   TGGTACAGCAGCCTGGAGAAAAGTCGTCAATGTGAAAACATCTATAGAGAAGGTCAACAC
161   W  Y  S  S  L  E  K  S  R  Q  C  E  N  I  Y  R  E  G  Q  H

541   ATTGCTTGTTCCTTTAAATTGACTAAAGTGGAACCTAGTTTTGAACATCAGAACGTTCAA
181   I  A  C  S  F  K  L  T  K  V  E  P  S  F  E  H  Q  N  V  Q

601   ATAATGGTCAAGGATAATGCTGGGAAAATTAGGCCATCCTGCAAAATAGTGTCTTTAACT
201   I  M  V  K  D  N  A  G  K  I  R  P  S  C  K  I  V  S  L  T

661   TCCTATGTGAAACCTGATCCTCCACATATTAAACATCTTCTCCTCAAAAATGGTGCCTTA
231   S  Y  V  K  P  D  P  P  H  I  K  H  L  L  L  K  N  G  A  L

721   TTAGTGCAGTGGAAGAATCCACAAAATTTTAGAAGCAGATGCTTAACTTATGAAGTGGAG
241   L  V  Q  W  K  N  P  Q  N  F  R  S  R  C  L  T  Y  E  V  E

781   GTCAATAATACTCAAACCGACCGACATAATATTTTAGAGGTTGAAGAGGACAAATGCCAG
261   V  N  N  T  Q  T  D  R  H  N  I  L  E  V  E  E  D  K  C  Q

841   AATTCCGAATCTGATAGAAACATGGAGGGTACAAGTTGTTTCCAACTCCCTGGTGTTCTT
281   N  S  E  S  D  R  N  M  E  G  T  S  C  F  Q  L  P  G  V  L

901   GCCGACGCTGTCTACACAGTCAGAGTAAGAGTCAAAACAAACAAGTTATGCTTTGATGAC
301   A  D  A  V  Y  T  V  R  V  R  V  K  T  N  K  L  C  F  D  D

961   AACAAACTGTGGAGTGATTGGAGTGAAGCACAGAGTATAGGTAAGGAGCAAAACTCCACC
321   N  K  L  W  S  D  W  S  E  A  Q  S  I  G  K  E  Q  N  S  T

1021  TTCTACACCACCATGTTACTCACCATTCCAGTCTTTGTCGCAGTGGCAGTCATAATCCTC
341   F  Y  T  T  M  L  L  T  I  P  V  F  V  A  V  A  V  I  I  L

1081  CTTTTTTACCTGAAAAGGCTTAAGATCATTATATTTCCTCCAATTCCTGATCCTGGCAAG
361   L  F  Y  L  K  R  L  K  I  I  I  F  P  P  I  P  D  P  G  K

1141  ATTTTTAAAGAAATGTTTGGAGACCAGAATGATGATACCCTGCACTGGAAGAAGTATGAC
381   I  F  K  E  M  F  G  D  Q  N  D  D  T  L  H  W  K  K  Y  D

1201  ATCTATGAGAAACAATCCAAAGAAGAAACGGATTCTGTAGTGCTGATAGAAAACCTGAAG
401   I  Y  E  K  Q  S  K  E  E  T  D  S  V  V  L  I  E  N  L  K

1261  AAAGCAGCTCCTTCAtggggagaagtgatttctttcttgccttcaatgtgaccctgtgaa
421   K  A  A  P  *

1321  gatttattgcattctccatttgttatctgggggacttgttaaatagaaactgaaactact
1381  cttgaaaaacaggcagctcctaagagccacaggtcttgatgtgacttttgcattgaaaac
1441  ccaaacccaaaggagctccttccaagaaaagcaagagttcttctcgttccttgttccaat
1501  ccctaaaagcagatgttttgccaaatccccaaactagaggacaaagacaaggggacaatg
1561  accatcaattcatctaatcaggaattgtgatggcttcctaaggaatctctgcttgctctg
```

FIGURE 1

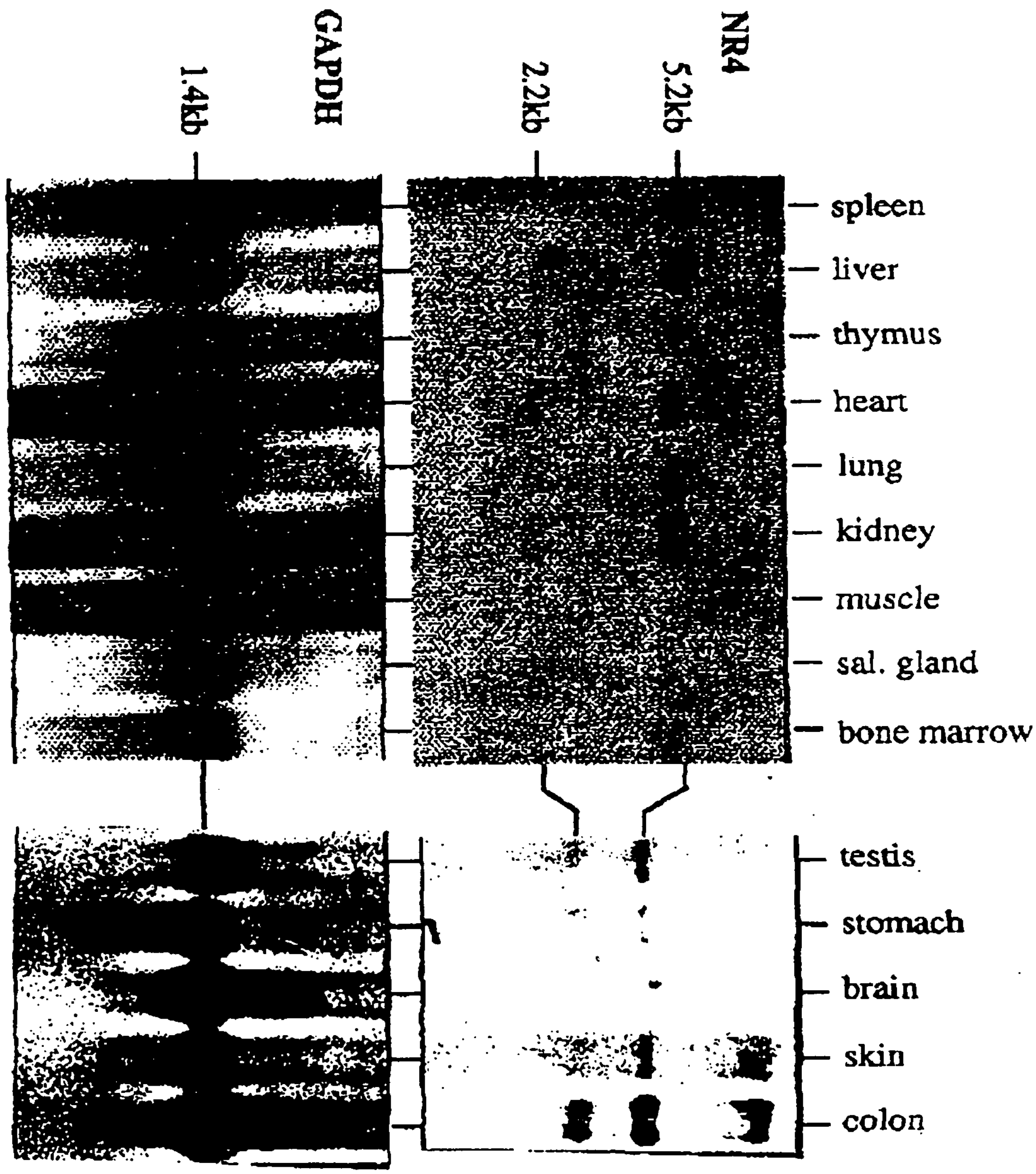

GAPDH
1.4kb
NR4
2.2kb
5.2kb
spleen
liver
thymus
heart
lung
kidney
muscle
sal. gland
bone marrow
testis
stomach
brain
skin
colon

FIGURE 2

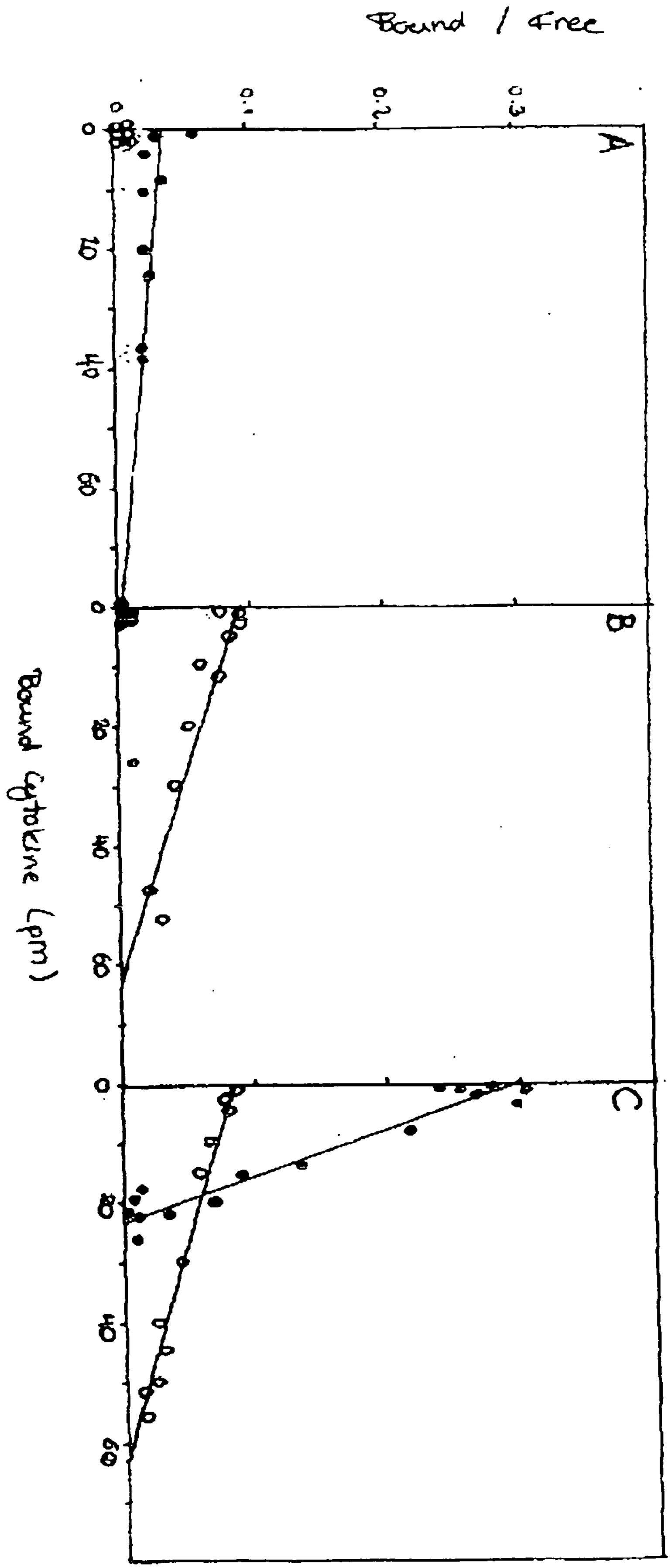



FIGURE 3

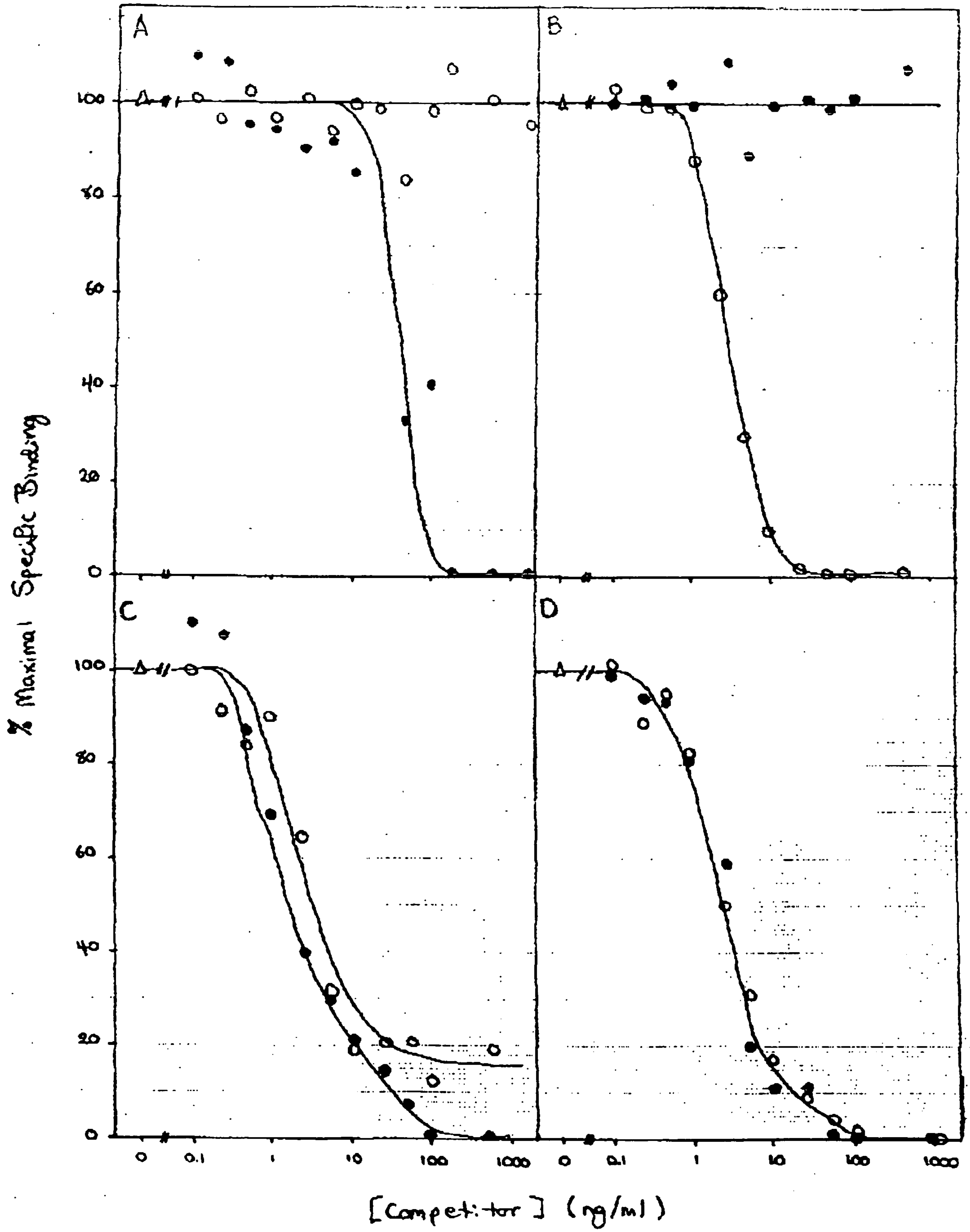

A
B
C
D
100
80
60
40
20
0
% Maximal Specific Binding
0
0.1
1
10
100
1000
[Competitor] (ng/ml)

FIGURE 4

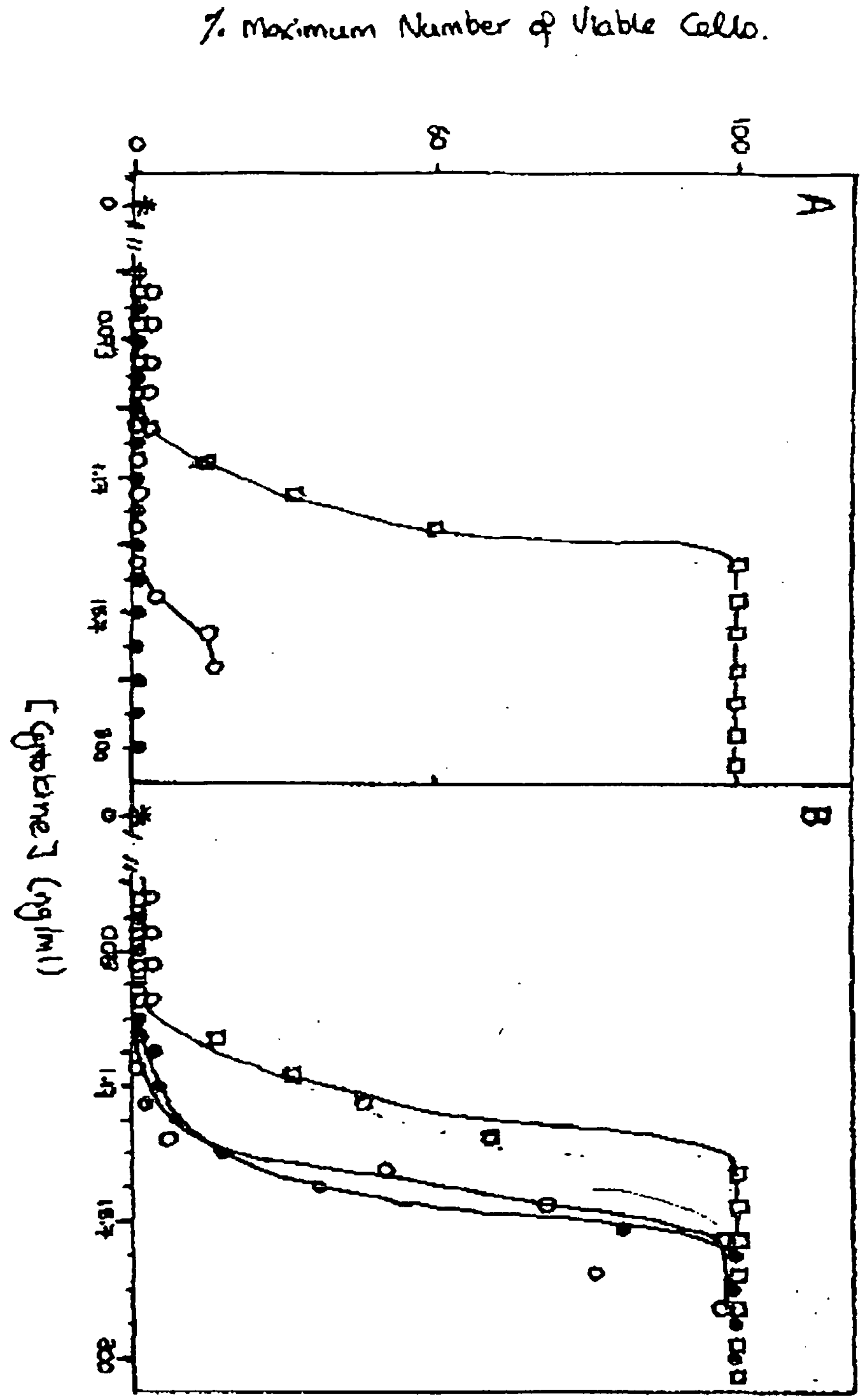
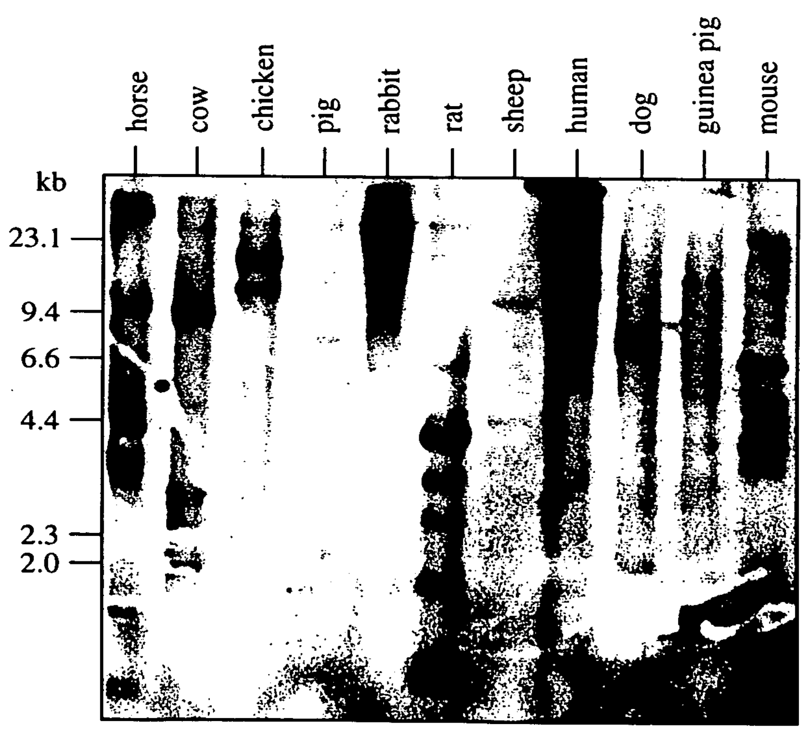



FIGURE 5

FIGURE 6

Cross-species conservation of the NR-4 (IL-13Rα) gene



# FIGURE 7

```
M  -60   tgaaaagatagaataaatggcctcgtgccgaattcggcacgagccgaggcgagggcctgc

M  1     ATGGCGCGGCCAGCGCTGCTGGGCGAGCTGTTGGTGCTGCTACTGTGGACCGCCACCGTG
M  1      M  A  R  P  A  L  L  G  E  L  L  V  L  L  L  W  T  A  T  V

H                     A  P  T  E  T  Q  P  P  V  T  N  L  S  V  S  V
H                    GCGCCTACGGAAACTCAGCCACCTGTGACAAATTTGAGTGTCTCTGTT
                      *     *  *     *  *  *  *  *  *  *  *  *  *  *
M  61    GGCCAAGTTGCCGCGGCCACAGAAGTTCAGCCACCTGTGACGAATTTGAGCGTCTCTGTC
M  21     G  Q  V  A  A  A  T  E  V  Q  P  P  V  T  N  L  S  V  S  V

H         E  N  L  C  T  V  I  W  T  W  N  P  P  E  G  A  S  S  N  C
H        GAAAACCTCTGCACAGTAATATGGACATGGAATCCACCCGAGGGAGCCAGCTCAAATTGT
          *  *  *  *  *     *  *  *  *     *  *  *  *  *  *     *  *
M  121   GAAAATCTCTGCACGATAATATGGACGTGGAGTCCTCCTGAAGGAGCCAGTCCAAATTGC
M  41     E  N  L  C  T  I  I  W  T  W  S  P  P  E  G  A  S  P  N  C

H         S  L  W  Y  F  S  H  F  G  D  K  Q  D  K  K  I  A  P  E  T
H        AGTCTATGGTATTTTAGTCATTTTGGCGACAAACAAGATAAGAAAATAGCTCCGGAAACT
             *     *  *  *  *  *     *     *  *  *  *  *  *  *  *  *
M  181   ACTCTCAGATATTTTAGTCACTTTGATGACCAACAGGATAAGAAAATTGCTCCAGAAACT
M  61     T  L  R  Y  F  S  H  F  D  D  Q  Q  D  K  K  I  A  P  E  T

H         R  R  S  I  E  V  P  L  N  E  R  I  C  L  Q  V  G  S  Q  C
H        CGTCGTTCAATAGAAGTACCCCTGAATGAGAGGATTTGTCTGCAAGTGGGGTCCCAGTGT
             *        *     *  *     *     *  *  *  *  *  *  *  *  *
M  241   CATCGTAAAGAGGAATTACCCCTGGATGAGAAAATCTGTCTGCAGGTGGGCTCTCAGTGT
M  81     H  R  K  E  E  L  P  L  D  E  K  I  C  L  Q  V  G  S  Q  C

H         S  T  N  E  S  E  K  P  S  I  L  V  E  K  C  I  S  P  P  E
H        AGCACCAATGAGAGTGAGAAGCCTAGCATTTTGGTTGAAAAATGCATCTCACCCCCAGAA
          *     *  *  *  *  *  *  *     *  *  *  *  *  *  *  *  *  *
M  301   AGTGCCAATGAAAGTGAGAAGCCTAGCCCTTTGGTGAAAAAGTGCATCTCACCCCCTGAA
M  101    S  A  N  E  S  E  K  P  S  P  L  V  K  K  C  I  S  P  P  E

H         G  D  P  E  S  A  V  T  E  L  Q  C  I  W  H  N  L  S  Y  M
H        GGTGATCCTGAGTCTGCTGTGACTGAACTTCAATGCATTTGGCACAACCTGAGCTACATG
          *  *  *  *  *  *  *  *  *  *     *  *  *  *  *  *  *  *  *
M  361   GGTGATCCTGAGTCCGCTGTGACTGAGCTCAAGTGCATTTGGCATAACCTGAGCTATATG
M  121    G  D  P  E  S  A  V  T  E  L  K  C  I  W  H  N  L  S  Y  M

H         K  C  S  W  L  P  G  R  N  T  S  P  D  T  N  Y  T  L  Y  Y
H        AAGTGTTCTTGGCTCCCTGGAAGGAATACCAGTCCCGACACTAACTATACTCTCTACTAT
          *  *  *  *  *  *  *  *  *  *  *  *  *  *     *  *  *  *  *
M  421   AAGTGTTCCTGGCTCCCTGGAAGGAATACAAGCCCTGACACACACTATACTCTGTACTAT
M  141    K  C  S  W  L  P  G  R  N  T  S  P  D  T  H  Y  T  L  Y  Y
```

## FIGURE 7 (continued...)

```
H          W  H  R  S  L  E  K  I  H  Q  C  E  N  I  F  R  E  G  Q  Y
H        TGGCACAGAAGCCTGGAAAAAATTCATCAATGTGAAAACATCTTTAGAGAAGGCCAATAC
          *        *  *  *  *        *  *  *  *  *     *  *  *  *
M 481    TGGTACAGCAGCCTGGAGAAAAGTCGTCAATGTGAAAACATCTATAGAGAAGGTCAACAC
M 161     W  Y  S  S  L  E  K  S  R  Q  C  E  N  I  Y  R  E  G  Q  H

H          F  G  C  S  F  D  L  T  K  V  K  D  S  S  F  E  Q  H  S  V
H        TTTGGTTGTTCCTTTGATCTGACCAAAGTGAAGGATTCCAGTTTTGAACAACACAGTGTC
                *  *  *     *  *  *  *           *  *  *           *
M 541    ATTGCTTGTTCCTTTAAATTGACTAAAGTGGAACCT---AGTTTTGAACATCAGAACGTT
M 181     I  A  C  S  F  K  L  T  K  V  E  P  -  S  F  E  H  Q  N  V

H          Q  I  M  V  K  D  N  A  G  K  I  K  P  S  F  N  I  V  P  L
H        CAAATAATGGTCAAGGATAATGCAGGAAAAATTAAACCATCCTTCAATATAGTGCCTTTA
          *  *  *  *  *  *  *  *  *  *  *     *  *        *  *     *
M 601    CAAATAATGGTCAAGGATAATGCTGGGAAAATTAGGCCATCCTGCAAAATAGTGTCTTTA
M 201     Q  I  M  V  K  D  N  A  G  K  I  R  P  S  C  K  I  V  S  L

H          T  S  R  V  K  P  D  P  P  H  I  K  N  L  S  F  H  N  D  D
H        ACTTCCCGTGTGAAACCTGATCCTCCACATATTAAAAACCTCTCCTTCCACAATGATGAC
          *  *     *  *  *  *  *  *  *  *  *     *           *
M 661    ACTTCCTATGTGAAACCTGATCCTCCACATATTAAACATCTTCTCCTCAAAAATGGTGCC
M 221     T  S  Y  V  K  P  D  P  P  H  I  K  H  L  L  L  K  N  G  A

H          L  Y  V  Q  W  E  N  P  Q  N  F  I  S  R  C  L  F  Y  E  V
H        CTATATGTGCAATGGGAGAATCCACAGAATTTTATTAGCAGATGCCTATTTTATGAAGTA
          *     *  *  *     *  *  *  *  *     *  *  *  *     *  *  *
M 721    TTATTAGTGCAGTGGAAGAATCCACAAAATTTTAGAAGCAGATGCTTAACTTATGAAGTG
M 241     L  L  V  Q  W  K  N  P  Q  N  F  R  S  R  C  L  T  Y  E  V

H          E  V  N  N  S  Q  T  E  T  H  N  V  F  Y  V  Q  E  A  K  C
H        GAAGTCAATAACAGCCAAACTGAGACACATAATGTTTTCTACGTCCAAGAGGCTAAATGT
          *  *  *  *     *  *        *  *           *     *     *  *
M 781    GAGGTCAATAATACTCAAACCGACCGACATAATATTTTAGAGGTTGAAGAGGACAAATGC
M 261     E  V  N  N  T  Q  T  D  R  H  N  I  L  E  V  E  E  D  K  C

H          E  N  P  E  F  E  R  N  V  E  N  T  S  C  F  M  V  P  G  V
H        GAGAATCCAGAATTTGAGAGAAATGTGGAGAATACATCTTGTTTCATGGTCCCTGGTGTT
             *     *        *  *     *     *  *  *  *        *  *  *
M 841    CAGAATTCCGAATCTGATAGAAACATGGAGGGTACAAGTTGTTTCCAACTCCCTGGTGTT
M 281     Q  N  S  E  S  D  R  N  M  E  G  T  S  C  F  Q  L  P  G  V

H          L  P  D  T  L  N  T  V  R  I  R  V  K  T  N  K  L  C  Y  E
H        CTTCCTGATACTTTGAACACAGTCAGAATAAGAGTCAAAACAAATAAGTTATGCTATGAG
          *     *           *  *  *     *  *  *  *  *  *  *  *
M 901    CTTGCCGACGCTGTCTACACAGTCAGAGTAAGAGTCAAAACAAACAAGTTATGCTTTGAT
M 301     L  A  D  A  V  Y  T  V  R  V  R  V  K  T  N  K  L  C  F  D

H          D  D  K  L  W  S  N  W  S  Q  E  M  S  I  G  K  K  R  N  S
H        GATGACAAACTCTGGAGTAATTGGAGCCAAGAAATGAGTATAGGTAAGAAGCGCAATTCC
          *     *  *  *  *     *  *           *  *  *  *        *  *
M 961    GACAACAAACTGTGGAGTGATTGGAGTGAAGCACAGAGTATAGGTAAGGAGCAAAACTCC
M 321     D  N  K  L  W  S  D  W  S  E  A  Q  S  I  G  K  E  Q  N  S
```

**FIGURE 7 (continued...)**

```
H          T  L  Y  I  T  M  L  L  I  V  P  V  I  V  A  G  A  I  I  V
H         ACACTCTACATAACCATGTTACTCATTGTTCCAGTCATCGTCGCAGGTGCAATCATAGTA
           *     *     *  *  *  *        *  *     *  *     *     *
M 1021    ACCTTCTACACCACCATGTTACTCACCATTCCAGTCTTTGTCGCAGTGGCAGTCATAATC
M 341      T  F  Y  T  T  M  L  L  T  I  P  V  F  V  A  V  A  V  I  I

H          L  L  L  Y  L  K  R  L  K  I  I  I  F  P  P  I  P  D  P  G
H         CTCCTGCTTTACCTAAAAAGGCTCAAGATTATTATATTCCCTCCAATTCCTGATCCTGGC
           *  *     *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
M 1081    CTCCTTTTTTACCTGAAAAGGCTTAAGATCATTATATTTCCTCCAATTCCTGATCCTGGC
M 361      L  L  F  Y  L  K  R  L  K  I  I  I  F  P  P  I  P  D  P  G

H          K  I  F  K  E  M  F  G  D  Q  N  D  D  T  L  H  W  K  K  Y
H         AAGATTTTTAAAGAAATGTTTGGAGACCAGAATGATGATACTCTGCACTGGAAGAAGTAC
           *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
M 1141    AAGATTTTTAAAGAAATGTTTGGAGACCAGAATGATGATACCCTGCACTGGAAGAAGTAT
M 381      K  I  F  K  E  M  F  G  D  Q  N  D  D  T  L  H  W  K  K  Y

H          D  I  Y  E  K  Q  T  K  E  E  T  D  S  V  V  L  I  E  N  L
H         GACATCTATGAGAAGCAAACCAAGGAGGAAACCGACTCTGTAGTGCTGATAGAAAACCTG
           *  *  *  *  *  *     *  *  *  *  *  *  *  *  *  *  *  *  *
M 1201    GACATCTATGAGAAACAATCCAAAGAAGAAACGGATTCTGTAGTGCTGATAGAAAACCTG
M 401      D  I  Y  E  K  Q  S  K  E  E  T  D  S  V  V  L  I  E  N  L

H          K  K  A  S  Q  *
H         AAGAAAGCCTCTCAGTGAtggagataatttatttttaccttcactgtgaccttgagaaga
           *  *  *
M 1261    AAGAAAGCAGCTCCTTGAtggggagaagtgatttctttcttgccttcaatgtgaccctgt
M 421      K  K  A  A  P  *
```

FIGURE 8

NR4 expression in mouse tissues